TECHNICAL BULLETIN NO. 235 OCTOBER, 1935

New York State Agricultural Experiment Station

Geneva, N. Y.

IMPERIAL MYCOLOGI
7 APR. 1936
INSTITUTE

THE PHYSIOLOGY OF BEAN MOSAIC

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PUBLISHED BY THE STATION
UNDER AUTHORITY OF CORNELL UNIVERSITY

ABSTRACT

THE investigation dealt with certain properties of the bean mosaic virus and with the effect of mosaic on the popular canning variety of *Phaseolus vulgaris,* the Stringless Green Pod Refugee.

The subjection of mosaic-infected seed to high temperatures, formaldehyde fumes, and X-rays failed to inactivate the virus or eliminate the infected seeds. Flotation methods failed to show any significant difference between the specific gravity of healthy and mosaic-infected bean seed.

Mosaic-infected plants have a lower percentage of dry matter than do healthy plants. The transpiration from mosaic-infected and healthy plants was studied by the use of auto-irrigators (Livingston and porous pot), balances, and cobalt chloride papers. Transpiration was significantly less per unit of surface area and per gram of dry matter from mosaic-infected plants than from healthy plants. Leaves with early symptoms transpired much more slowly than leaves with typical symptoms as measured by cobalt chloride papers. The same method demonstrated that the light green areas transpired more slowly than the dark green areas. The fact that fewer stomates are open on mosaic-infected leaves than on healthy leaves probably accounts for the reduction in the rate of transpiration from mosaic-infected plants. The ratio of the transpiration rates from detached mosaic-infected and healthy leaves is the same as that from attached leaves.

Detached pods from mosaic-infected plants lose weight faster per unit of surface area and per gram of fresh weight than detached pods of the same size from healthy plants. The increased rate of transpiration from mosaic-infected pods may be explained on the basis that the diseased pods are unable to hold water as well as the healthy pods.

Altho mosaic-infected plants produce fewer blooms per plant than healthy plants, they produce more blooms per gram of dry matter. A lower percentage of the blooms, however, set on infected plants than on healthy plants. The reduction in yield from mosaic-infected plants is attributed to the stunting effect of the disease, since mosaic-infected and healthy plants of the same size produce approximately the same number of pods. Bean mosaic causes a delay of several days in the time of blooming, consequently infected plants remain green longer than do healthy plants.

Bean mosaic frequently causes the production of curved, misshapen, hydrotic, and, on plants infected just prior to or during the blooming period, rough, edematous pods.

Symptoms of mosaic tend to be masked at temperatures of 30°C or above and of 15°C or below. In a favorable temperature and with the light intensity of more than 800 foot-candles, typical symptoms of bean mosaic developed when exposed to 8, 10, or 12 hours of light daily. At intensities of less than 300 foot-candles the symptoms were very faint.

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THE PHYSIOLOGY OF BEAN MOSAIC[1]

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INTRODUCTION

Bean mosaic was first recognized by Iwanowski (19)[3] in 1899. Little attention, however, was given to the disease until after the severe epithytotics of 1916 and 1917 which almost annihilated the pea bean industry in certain sections of the United States. General phases of bean mosaic have been studied by Stewart and Reddick (47), Reddick and Stewart (41 and 42), Reddick (43), Fajardo (11, 12), Pierce and Hungerford (37), Nelson (34, 35), Zaumeyer and Wade (52), and Pierce (36); but no detailed study has been made of the effect of mosaic on the physiologic activities of the bean, such as transpiration, assimilation, and reproduction, which have been subjects for the investigations reported herein. Attempts were also made to eliminate the virus from infected seeds by heat, specific gravity, chemicals, and X-rays.

MATERIALS AND METHODS

The investigations were confined to the one variety Stringless Green Pod Refugee because of its importance to the bean canning industry of New York State and because this variety is highly susceptible to the common bean mosaic. Emphasis on one variety permitted a more detailed study of the physiology of the disease.

Seeds for the investigations were obtained from different sources.

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[1] Condensed from a thesis presented to the Faculty of the Graduate School of Cornell University, June, 1935, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to acknowledge his indebtedness to Professor James G. Horsfall and Professor W. H. Burkholder for valuable assistance and advice during the investigations and helpful criticism in preparation of the manuscript; and to the Rogers Bros. Seed Co., Chicago, Ill., which sponsored the fellowship and so made these investigations possible.

[3] Refers to Literature Cited, page 45.

The pure lines of mosaic-infected seeds used in the experiments were obtained from Dr. J. G. Horsfall of this Station. The mosaic-free seed used in the experiments was obtained either by field roguing in the fall or from healthy plants grown in cheesecloth cages.

The large cages used in the field experiments were constructed with a wooden frame, approximately 32 feet long, 5 feet wide, and 3.5 feet high, over which was stretched cheesecloth. This cheesecloth was of a good grade, having 40 x 44 threads per inch. Cages could be opened at the end for entering. They proved very satisfactory for growing plants to maturity.

In the greenhouse experiments the plants were usually grown in electrically pasteurized soil (18), since beans appeared to do better in soil treated in this way than in steamed soil or in untreated soil.

Student's method was used in calculating the mathematical significance of the data presented herein. The odds were obtained from Love's (30) modification of Student's "one-tailed" table. Consequently, odds of 40:1 were considered necessary for significance.

SYMPTOMS

The fact that beans are susceptible to more than one virus was first discovered by Carsner (3). Later, Wingard (49) demonstrated that the tobacco ring spot virus may infect beans. Price (39) demonstrated that the tobacco mosaic virus causes local lesions on the bean. Zaumeyer (51) reports transmission of various legume mosaics to *Phaseolus vulgaris,* but whether or not he dealt with the virus of the common bean mosaic is not certain since he states that in some cases the symptoms were not typical. Merkel (32) concluded that one virus was responsible for all the mosaic diseases of the Leguminosae. Fajardo (11) believed that there was only one type of bean mosaic. Nelson (35), on the basis of symtom expression, described a disease of beans which he referred to as "rugose mosaic". The work of Pierce (36) has demonstrated clearly that there are at least two distinct mosaic diseases occurring naturally on the bean. He refers to them as the common bean mosaic and the yellow bean mosaic. The latter is not seed borne.

Investigations reported herein have dealt exclusively with the common bean mosaic. The symptoms of this mosaic on the variety Stringless Green Pod Refugee vary greatly under different conditions.

In general, bean mosaic causes a mottling and downward rolling of the leaf margins. The leaf symptoms usually are most severe on

seedlings arising from infected seeds and on plants recently infected with the virus. They become less pronounced as the season progresses. Frequently, vines proliferate markedly giving a bushy plant. Mosaic stunts the plants, especially under poor growing conditions. The writer (16) has presented a more complete description of the symptoms of bean mosaic on the variety Stringless Green Pod Refugee in another publication. However, pod and flower symptoms and the rôle that climate plays in the development of symptoms are described in other sections of this paper.

RESISTANCE OF THE VIRUS IN THE SEED TO ELIMINATION

Several attempts were made to eliminate the mosaic virus from infected bean seeds by subjecting the seeds to heat under various conditions, to formaldehyde fumes, and to X-rays. Specific gravity also was tried as a method of separating the mosaic-infected from the healthy seeds.

The incidence of mosaic was determined in all cases by planting the treated seeds in flats of steamed soil or electrically pasteurized soil (18) in the greenhouse. Records on the germination and mosaic content were taken when the first compound leaf had fully expanded.

Several workers (42, 12) report attempts to eliminate the virus from bean seed by heat but with negative results. No mention is made, however, of the moisture content of the seed at the beginning of the treatment. That moisture is of prime importance in determining the amount of heat that various seeds will withstand without loss of viability has been conclusively demonstrated by Dixon (8), Lehman (24), and others. Also, Lipscomb and Corley (25) have shown that cotton seeds will withstand higher temperatures in vacuum or in an inert gas than in an ordinary oven.

EXPERIMENTS

The experiments to eliminate the virus by heat were conducted by placing the samples in small beakers in a thermo-regulated Freas electric oven. In all cases the seeds were pre-dried in an electric paraffin oven at 40°, 50° or 60°C, but more frequently at 50°C.

In one experiment the pre-dried seeds were subjected to heat under high humidity by placing the small beakers in jars with ground-glass stoppers. In each jar was a small amount of water to maintain the high humidity. After treatment, all samples which had been subjected

to drying conditions were hand scarified to insure germination. A summary of the results of the experiments with heat are presented in Table 1.

TABLE 1.—THE THERMAL INACTIVATION POINT OF SEED AND MOSAIC VIRUS WHEN TREATED WITH HEAT UNDER DRY AND MOIST CONDITIONS.

TREATMENT		DRY CONDITIONS		MOIST CONDITIONS	
°C	Duration	Germination, per cent	Mosaic, per cent	Germination, per cent	Mosaic, per cent
100°	6 hrs.	46	52.1	52	46.1
100°	12 hrs.	24	45.4	8	50.0
100°	24 hrs.	28	57.1	0	0.0
100°	48 hrs.	2	0.0	0	0.0
None		94	51.0	94	51.0
90°	4½ hrs.	100	41.0	—	—
90°	5 hrs.	55	50.9	—	—
100°	4½ hrs.	97	29.9	—	—
100°	5 hrs.	25	24.0	—	—
None		98	38.8	—	—
90°	4½ hrs.	97	57.7	—	—
100°	3½ hrs.	96	42.7	—	—
100°	4 hrs.	35	45.7	—	—
100°	4½ hrs.	0	0.0	—	—
None		99	56.6	—	—
120°	10 min.	98	40.4	—	—
120°	20 min.	20	20.0	—	—
120°	30 min.	0	0.0	—	—
None		98	59.2	—	—
120°	10 min.	44	50.0	—	—
120°	20 min.	10	60.0	—	—
120°	30 min.	0	0.0	—	—
None		50	60.0	—	—
120°	30 min.*	26	46.2	—	—
120°	50 min.*	10	40.0	—	—
130°	10 min.*	100	42.0	—	—
130°	20 min.*	38	36.8	—	—
None		96	50.0	—	—

*Treatments in 25 inches of mercury vacuum.

These data show that pre-dried bean seeds will withstand higher temperatures and much longer exposures without injury than those seeds not pre-dried. Some seeds survived treatments of several hours at 100°C, while others survived at 120°C for 20 minutes. In 25 inches of mercury vacuum some of the seeds survived exposures of 120°C for 50 minutes and 130°C for 20 minutes.

Under conditions of high humidity, however, the seeds were killed with much shorter exposures than under conditions of low humidity. Mosaic was present in all samples which germinated.

In one experiment an effort was made to determine whether the virus was still active in seeds killed by high temperatures. Young healthy bean plants were inoculated with the macerated seeds which had been treated at 120°C for different lengths of time. Infection resulted only in the series in which some of the seeds were still viable. This experiment indicates that the thermal inactivation point of the virus is very close to that of the seed.

In experiments with formaldehyde the mosaic-infected seeds were subjected to the fumes for varying lengths of time by suspending the seeds in a wire basket over commercial formalin. The moisture content of the seeds determined to a large extent the length of exposure which they would withstand without loss of viability. Thirty-six per cent of the seeds (moisture content approximately 12 per cent) from warehouse storage germinated after 16 days exposure to the fumes, whereas only 3 per cent of the seeds with a moisture content of 20.8 per cent germinated after 3 hours exposure.

As in the case of heat treatments, mosaic was present in all samples which germinated.

In the X-ray experiments, 25 seeds were subjected to each of the following exposures: 1,000, 2,000, 4,000, 8,000, 16,000 and 32,000 R-units[4]. No injury was evident on the young plants grown from seeds treated with the 1,000 and 2,000 R-units, but it was progressively more serious beginning with the 4,000 R-units treatment. The plants grown from the seeds treated with 32,000 R-units were severely stunted and distorted, many of the plants dying in the seedling stages. The injury illustrated in Fig. 1 was not transmitted to the progeny of the injured plants. Mosaic was present in all samples.

Since mosaic was known to affect bean growth, it was thought that the disease might change the specific gravity of the seed; hence two experiments were made in attempts to separate the mosaic-infected from the healthy seeds on the basis of specific gravity. The seeds were immersed in solutions of potassium carbonate with different specific gravities. In the first experiment three different strengths and in the second four different strengths were used. There was no significant difference in the incidence of mosaic in the different samples.

Since the air pocket between the cotyledons varies greatly in different seeds, the above experiments gave no information as to whether

[4] The treatments were made thru the courtesy of Dr. L. F. Randolph of Cornell University.

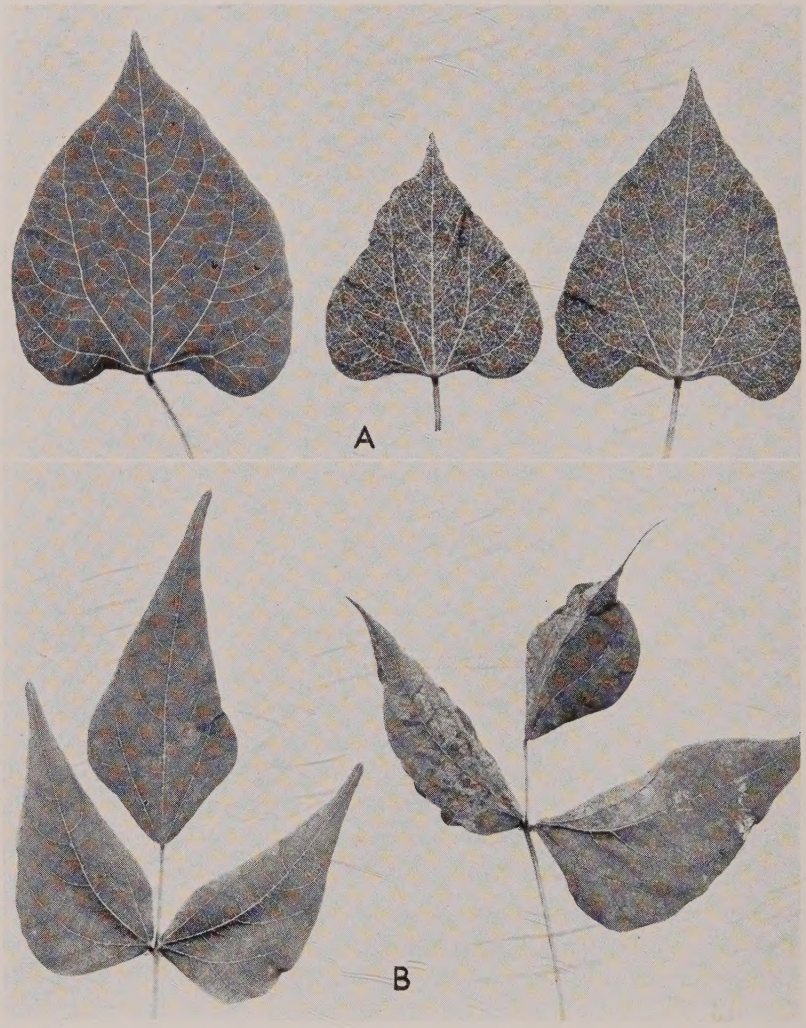

FIG. 1.—X-RAY INJURY ON SEEDLING LEAVES GROWN FROM SEED SUBJECTED TO 32,000 R-UNITS.

A, simple leaves; B, compound leaves. Check leaves at left.

the density of mosaic-infected cotyledons was greater or less than the density of healthy cotyledons. To test this, seeds were indexed in the greenhouse by cutting them in half. The half containing the plumule and radicle was planted in pasteurized soil to determine the presence or absence of mosaic. The remaining mosaic-infected and

healthy cotyledons thus obtained were immersed in a solution of calcium chloride with a specific gravity of 1.368, but no significant difference was observed in the densities of the healthy and the diseased cotyledons.

DISCUSSION

The results from the experiments cited in the preceding paragraphs prove that bean mosaic is extremely difficult to inactivate while in association with the living seed. The observation of Pierce and Hungerford (38) that the virus remained active in a sample of bean seed for 30 years supports this conclusion.

Other viruses, as for instance potato mosaic and leafroll (1) and sugar cane mosaic (13), are extremely difficult to inactivate while in association with the living tissues. Wilbrink (48), on the other hand, claims to have eliminated the causal agent of sereh disease of sugar cane by heat treatments. Also Kunkel (22) has demonstrated recently that the viruses of peach yellows and little peach were inactivated by exposing the growing trees to a temperature of 35° to 37°C for a period of 2 weeks.

EFFECT OF MOSAIC ON THE PHYSIOLOGY OF THE PLANT

ASSIMILATION

The metabolism of virus-infected plants varies greatly from the normal. Certain viruses cause an accumulation of certain metabolic products, while others cause depletion. For a review of the literature dealing with this subject the reader is referred to Smith (45).

Altho no work has been done on the effect of mosaic on the metabolism of the bean, time did not permit a detailed study of this subject. Dry matter determinations, however, were made of mosaic-infected and healthy bean plants during the course of the investigations.

Samples were collected for the dry matter determinations from plantings of a pure line of seed infected with mosaic during the summers of 1932, 1933, and 1934. The roots were removed as soon as possible and the plants dried at 100°C.

In 1932, three samples of 30 plants each were collected from weekly plantings in the field when in the first compound-leaf stage and divided into three classes, *viz.*, mosaic, healthy, and doubtful. For the 1933 dry matter determinations, three samples of 15 mosaic-infected and 15 healthy plants each were gathered from each of the weekly plantings in the field. The plants were in the first compound-leaf stage.

The samples for the dry matter determinations in 1934 were obtained from greenhouse plantings. The samples contained from 5 to 35 plants, depending on the size and number of plants available. The same number of plants, however, were used in each sample for each determination. Usually three samples each of mosaic-infected and healthy plants were used for each determination. The plants varied in age from seedlings to plants in bloom.

The results, a summary of which is presented in Table 2, prove that mosaic-infected plants have a lower percentage of dry matter than healthy plants. The fact that the dry matter content is lower in the mosaic-infected plants is not surprising, since many workers, including Dickson (7), Elmer (10), Sorokin (46), and Eckerson (9), have shown that in virus diseases the chlorophyll and chloroplasts are greatly altered.

TABLE 2.—EFFECT OF MOSAIC ON DRY MATTER CONTENT OF PLANTS.

No. of test	Average percentage dry matter					
	Series I*		Series II†		Series III‡	
	Healthy	Mosaic	Healthy	Mosaic	Healthy	Mosaic
1	16.9	15.9	22.7	21.5	11.0	10.6
2	17.0	16.5	19.1	19.2	9.8	9.5
3	15.8	14.0	21.7	20.7	10.7	9.8
4	15.7	14.1	19.2	19.1	10.8	10.7
5	14.8	13.5	17.4	17.3	12.8	12.5
6	14.5	14.2	19.1	17.6	13.6	13.6
7	14.5	14.1	—	—	11.1	10.9
8	14.2	13.8	—	—	12.6	12.0
9	16.0	14.6	—	—	13.3	13.1
10	15.9	14.9	—	—	12.7	12.9
11	16.6	15.7	—	—	10.9	10.8
12	16.9	16.0	—	—	9.4	9.1
Average	15.7	14.8	19.9	19.2	11.6	11.3
Per cent decrease		5.7		3.5		2.6
Odds		10,000:1		25:1		293:1

*Determinations made on plants collected from field plantings in the summer of 1932.
†Determinations made on plants collected from field plantings in the summer of 1933.
‡Determinations made on plants collected from greenhouse plantings in the summer of 1934.

The decreased rate of transpiration from mosaic-infected plants, which is discussed later in this paper, may also partly account for the decreased percentage of dry matter.

TRANSPIRATION

The effect of fungi on the rate of transpiration of their suscepts has

been investigated by many workers, but little attention has been given to a study of the effect of viruses on the rate of transpiration of their suscepts.

Schander and associates (44) and Krüger (21) showed that potato plants affected with leafroll transpire less than healthy plants under some conditions and more than healthy plants under other conditions. Heuberger and Norton (17) observed that the transpiration rate of tomato plants affected with mosaic was higher than that of healthy plants. A sharp drop in the rate of transpiration occurs coincidentally with the appearance of the first visible symptoms, to be followed by a gradual increase in the transpiration rate. Kuprewicz (23) states that the transpiration rate of potatoes affected with mosaic is nearly doubled as compared with healthy plants, while in the case of potatoes affected with leafroll the transpiration rate approaches the normal.

Transpiration from pods.—The experiments on the effect of bean mosaic on the rate of transpiration were begun in the fall of 1933 as the result of an observation by Dr. W. H. Burkholder of Cornell University to the effect that mosaic-infected pods wilted much faster than healthy pods. This point of differential wilting is of importance not only to bean canners, but to all growers and shippers of snap beans as well, since the quality of mosaic-infected pods deteriorates more rapidly than that of the healthy pods. Hence, mosaic-infected beans cannot be held as long before being canned as can healthy pods.

This differential in the wilting rate may be based on a difference in the transpiration rate. Therefore, a series of experiments was conducted to study the effect of mosaic on the rate of transpiration from pods. For this purpose pods were picked from mosaic-infected and healthy plants of the Stringless Green Pod Refugee in the greenhouse and in the field; also, for one experiment, from the Corbett Refugee which is immune to mosaic.

All samples of Stringless Green Pod Refugee were spread out one layer deep on a laboratory table and weighed at frequent intervals to determine the water loss. The samples of Corbett Refugee were dried slowly in a chamber at 35°C and weighed at intervals for the same purpose. In some of these experiments the surface area of the pods was calculated on the basis of a cylinder, since the pods were approximately cylindrical. The volume was obtained by immersion in water.

The experiment with the Corbett Refugee pods was conducted to determine what effect size of the pod has on the rate of transpiration. The results showed that the small pods transpired faster per unit of

surface area and per gram of fresh weight than did the large pods.

In the next experiment the age of the pods was used as a basis for comparing the rate of water loss for diseased and healthy pods. But age alone was not a reliable basis for comparison, as is illustrated in Table 3 and in Figs. 2 and 3, since size apparently is of more importance in determining the rate of water loss than is age. This is demonstrated in the case of the 13- and 14-day-old diseased pods. The younger pods were the larger and therefore transpired more slowly than did the older pods.

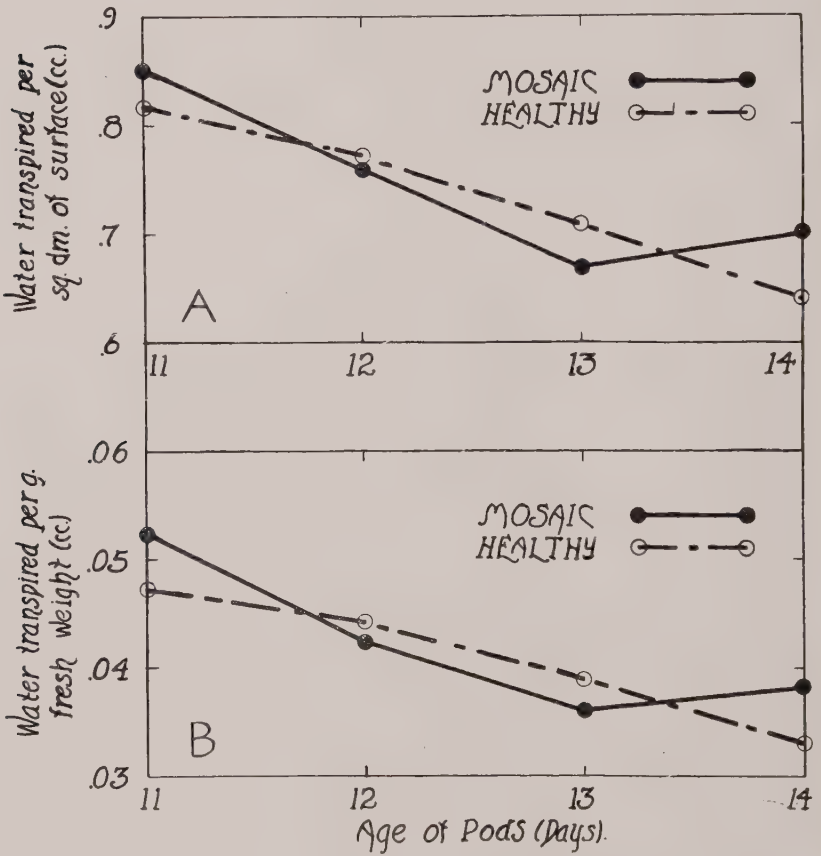

FIG. 2.—THE AMOUNT OF WATER TRANSPIRED IN 7 HOURS FROM MOSAIC-INFECTED AND HEALTHY PODS OF DIFFERENT AGES.

A, water transpired per unit of surface area; B, water transpired per unit of fresh weight. Data from Table 3.

Since age proved unreliable as a basis for comparison the data were replotted on the basis of pod size. The curves presented in Fig. 3 show that mosaic-infected pods transpire faster per unit of surface area and per gram of fresh weight than do healthy pods of the same size.

Additional data showing that mosaic-infected pods transpire faster than do healthy pods of the same size were obtained in another experiment in which the pods were graded with a home-made grader according to the sizes used by canners. A representative sample of the results is presented in Fig. 4. A greater variation in size of the pods was used in this experiment, but the results were the same from large pods as from very small pods. The rate of transpiration per square centimeter of surface was not calculated in this experiment,

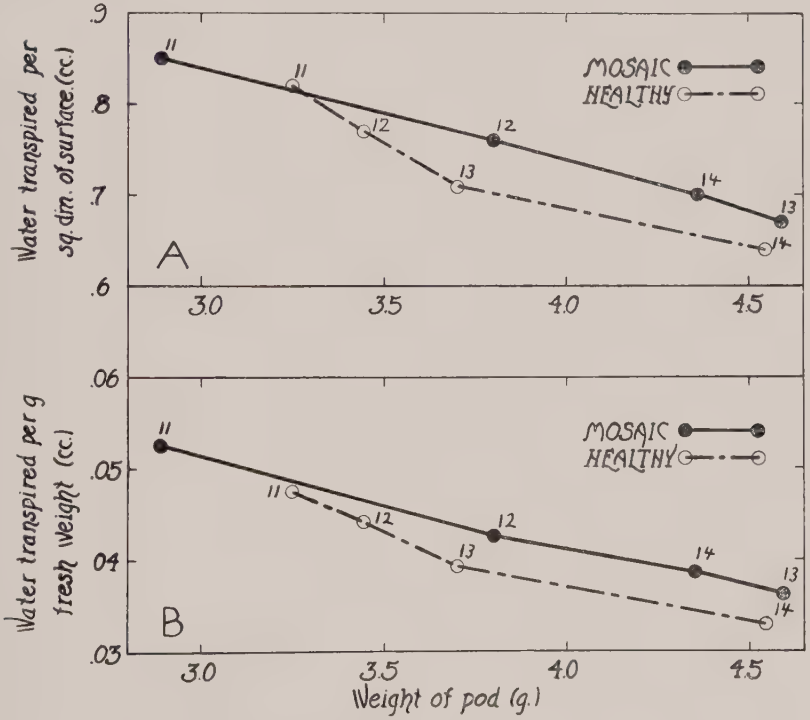

FIG. 3.—RELATION BETWEEN TRANSPIRATION AND SIZE OF PODS FROM DISEASED AND HEALTHY PODS.

A, water transpired per unit of surface area; B, water transpired per unit of fresh weight. Data from Table 3. Figures beside the points give the age of the pods in days.

TABLE 3.—WATER LOST IN SEVEN HOURS FROM DETACHED MOSAIC-INFECTED AND HEALTHY PODS OF DIFFERENT AGES.

	CONDITION	AGE OF PODS IN DAYS			
		11	12	13	14
Average weight per pod, grams	Healthy	3.25	3.44	3.70	4.54
	Mosaic	2.88	3.80	4.58	4.36
Surface area per pod, sq. cm.	Healthy	18.76	19.68	20.49	23.41
	Mosaic	17.74	21.47	24.81	23.82
Water lost per pod, grams	Healthy	0.154	0.151	0.146	0.150
	Mosaic	0.151	0.162	0.166	0.168
Water lost per gram of fresh weight, grams	Healthy	0.047	0.044	0.039	0.033
	Mosaic	0.052	0.043	0.036	0.039
Water lost per sq. dm. of surface, grams	Healthy	0.82	0.77	0.71	0.64
	Mosaic	0.85	0.75	0.67	0.71

since in previous experiments the same general type of curve was obtained whether the calculations were based on surface area or on fresh weight of the pod (Figs. 2 and 3).

Transpiration from leaves.—A quick, accurate method for measuring the area of bean leaves was needed before extensive experiments could be conducted to study the effect of mosaic on transpiration from leaves. Neither the method of Caruthers (4) nor that of Marshall

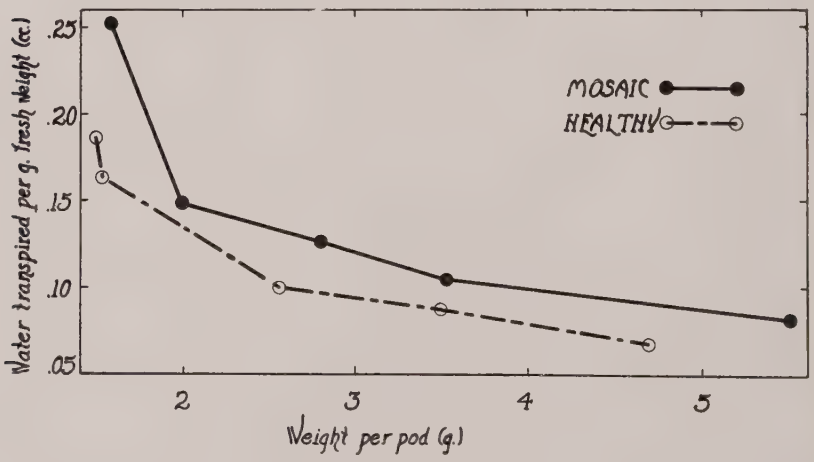

FIG. 4.—RELATION BETWEEN TRANSPIRATION AND SIZE OF PODS FROM DISEASED AND HEALTHY PODS.

Pods grouped according to canners' sieve sizes.

(31) appeared to be suited to the conditions of these experiments. A method employed by Darrow (6) for measuring the area of strawberry leaves and by Graf-Marin (14) for measuring the area of barley leaves, however, proved to be satisfactory and rapid. This method consisted of multiplying the product of the length and width of the leaf by the regression factor which is calculated from the correlation coefficient of the product and the area. The accuracy of this method when applied to bean leaves was checked by measuring blue prints of mosaic-infected and healthy leaves. These calculations (Table 4) show that a very close relationship exists between the product of the width and length and the area as measured by a planimeter. Furthermore, this relationship holds for both mosaic-infected and healthy leaves. A total of 299 simple leaves and leaflets was used in these calculations. The error of a single determination ranged from 3.4 to 8.6 per cent. This error, however, was greatly reduced, usually to less than 1 per cent, when a large number of measurements was considered. The method was tried only on the variety Stringless Green Pod Refugee.

Table 4.—The Accuracy of a Short Method to Obtain the Area of Bean Leaves.*

Type of leaf	Correlation co-efficient between area and product of width and length	Regression factor	Percentage error single determination	
			Healthy	Mosaic
Simple	.96±.0081	.7776	8.6	3.4
Apical leaflets of trifoliate leaves	.99±.0019	.5403	4.7	4.1
Lateral leaflets of trifoliate leaves	.99±.0013	.5967	4.6	3.6

*The method used was to multiply the product of the width and length of the leaf or leaflet by the corresponding regression factor.

Experiments to measure the amount of water transpired from mosaic-infected and healthy plants were conducted during the summer of 1934, using Livingston auto-irrigators (26), porous pot auto-irrigators[5], and balances.

Livingston (26, 27) states that there are individual differences between the porous cups, which probably is true also for the porous pot auto-irrigators. These possible individual differences did not seem

[5] These irrigators were designed by Dr. James G. Horsfall of this Station.

great enough, however, to warrant standardization of each auto-irrigator, since the results of several were to be averaged together.

The Livingston auto-irrigators were placed in 1-gallon glazed crocks, one to each crock.

The porous pot auto-irrigators used were similar to the Wilson (50) auto-irrigators. They consisted, in brief, of a 6-inch porous pot, minus the hole, sealed air-tight into a special 1-gallon glazed crock so that a space for water remained between the outer wall of the pot and the inner wall of the crock. Water used by the plant was drawn by capillary tension thru the walls of the porous pot from a reservoir. Thus, by measuring the water removed from the reservoir, the amount of water used by the plant in the auto-irrigator was obtained. A view of some of the auto-irrigators used in the second experiment is given in Fig. 5.

The Livingston auto-irrigators were used only in the first experiment, since the porous pot auto-irrigators proved to be more satisfactory because they have a greater moisture-supplying surface and probably maintain a more uniform moisture content thruout the soil.

FIG. 5.—POROUS POT AUTO-IRRIGATORS USED IN THE SECOND TRANSPIRATION EXPERIMENT.

Note the soil moisture recorder in the left background. The sensitive unit is embedded in the second pot from the left in the back row.

Furthermore, any air from leakage in the system can be removed easily at any time during the course of the experiment.

The crocks and auto-irrigators were sterilized with formaldehyde, filled with thoroly-mixed, electrically-pasteurized soil, and planted with a pure line of seed infected with mosaic. As soon as the plants were large enough for mosaic to be detected, they were thinned to one in each container. The surface of the soil was then covered with a sealing mixture (beeswax 1 part and paraffin 4 parts) to prevent the loss of water at that point. This mixture is a modification of the Briggs and Shantz (2) mixture. It proved to be fairly satisfactory except during periods of extreme fluctuations in temperature, when it cracked away from the edge of the crocks. These cracks, however, were resealed with a hot instrument.

The reservoirs of water were placed about 6 inches below the level of the auto-irrigators, making the capillary tension of the system so slight that the soil remained very moist. A check on the moisture content of one of the porous pot auto-irrigators was obtained in one of the series by using a soil moisture recorder designed by Dr. James G. Horsfall. It showed that the maximum capillary tension did not vary from day to day, but stayed at 20 mm of mercury. The moisture recorder is shown in the background of Fig. 5. The sensitive unit is buried in the second pot from the left in the back row.

In the first experiment employing 9 Livingston and 14 porous pot auto-irrigators, the leaf measurements were made at weekly intervals, commencing May 19, while daily readings on the amount of water transpired were taken each morning as near 8:30 as possible. Each leaf-area measurement was used as the basis for calculating the water transpired per unit of area for the 4 days preceding and the 3 days succeeding the measurement. The first compound leaf was partly expanded at the beginning of the experiment, which continued for 21 days when some of the plants began to bloom.

The need for protection from the hot rays of the sun was evident on May 22 when three leaves were injured because of excessive transpiration. The injured leaves were removed and a cheesecloth shade erected over the plants. No more injury to the leaves resulted in this experiment.

A series of 14 porous pot auto-irrigators was used in the second experiment and the plants were shaded by cheesecloth from the hot rays of the sun.

Since a lapse of 7 days appeared to be too long between measurements because of the rapid increase in the total leaf surface, the

leaves were measured every 4 days. Readings on the amount of water transpired were made every 2 days as near 8:30 in the morning as possible. Thus, each leaf-area measurement was used as the basis for calculating the amount of water transpired for the 2 days preceding and the 2 days succeeding the measurement. A view of some of the auto-irrigators used in this experiment is presented in Fig. 5.

The third experiment, in which 12 tared 1-gallon crocks were weighed to obtain the amount of water transpired, was run coincidentally with the second. Unfortunately, the plants were not protected from the hot rays of the sun and on September 3 some of the leaves on the healthy plants were injured because of excessive transpiration. The injured leaves were removed as were also a like number from the mosaic-infected plants, altho none of the leaves on these plants

TABLE 5.—CUBIC CENTIMETERS OF WATER TRANSPIRED PER SQUARE CENTIMETER OF LEAF SURFACE FROM MOSAIC-INFECTED AND HEALTHY PLANTS.

DAY	WATER TRANSPIRED PER SQ. CM. OF LEAF SURFACE IN CC*			
	Livingston auto-irrigators		Porous pot auto-irrigators	
	Healthy, av. of 6 plants	Mosaic, av. of 7 plants	Healthy, av. of 3 plants	Mosaic, av. of 5 plants
May 20	0.208	0.197	0.173	0.145
May 21	0.256	0.236	0.227	0.190
May 22	0.349	0.310	0.335	0.272
May 23	0.145	0.114	0.130	0.118
May 24	0.147	0.123	0.124	0.107
May 25	0.125	0.104	0.111	0.093
May 26	0.130	0.109	0.110	0.101
May 27	0.125	0.104	0.095	0.090
May 28	0.161	0.139	0.155	0.142
May 29	0.167	0.142	0.164	0.157
May 30	0.122	0.108	0.124	0.114
May 31	0.144	0.129	0.162	0.150
June 1	0.138	0.120	0.148	0.137
June 2	0.158	0.137	0.160	0.148
June 3	0.263	0.234	0.271	0.269
June 4	0.243	0.211	0.245	0.235
June 5	0.282	0.249	0.264	0.263
June 6	0.146	0.128	0.163	0.155
June 7	0.148	0.131	0.163	0.160
June 8	0.092	0.087	0.103	0.104
June 9	0.106	0.094	0.095	0.087
Average	0.174	0.153	0.168	0.154
Percentage reduction in transpiration		12.1		8.3
Odds	10,000:1		9,999:1	

*The leaves were measured on May 19 and 26 and June 2 and 9.

were injured. A cheesecloth shade similar to the one used in the other experiments was then erected over these plants. No more measurements nor readings were made for several days in order to allow the plants to recover from this severe shock.

The effect of mosaic on the rate of transpiration was determined also with cobalt chloride paper clips, which were prepared according to the method of Meyer (33).

The readings always were made just before or just after 12:00 o'clock noon. A stop watch was employed to check the time required to change the cobalt chloride paper from blue to bluish pink, the end point being determined by comparison with a standard piece of colored paper. The clips were fastened near the apex of the terminal leaflet of the trifoliate leaf except when comparisons were made on the rate of transpiration between the light and dark green areas of mosaic-infected leaves. Readings were made only on the lower surface of the leaf, only on leaves of approximately the same age and size, and only under similar growing conditions.

The results from experiments with auto-irrigators and balances show that the mosaic-infected plants transpire significantly less per

TABLE 6.—CUBIC CENTIMETERS OF WATER TRANSPIRED PER SQUARE CENTIMETER OF LEAF SURFACE FROM MOSAIC-INFECTED AND HEALTHY PLANTS.

PERIOD OF TIME	WATER TRANSPIRED PER SQ. CM. OF LEAF SURFACE IN CC*			
	Porous pot auto-irrigators		Weighing method	
	Healthy, average of 6 plants	Mosaic, average of 7 plants	Healthy, average of 5 plants	Mosaic, average of 6 plants
Aug. 28–30	0.250	0.224	——	——
Aug. 30–Sept. 1	0.271	0.242	——	——
Sept. 1–3	0.216	0.203	0.439	0.388
Sept. 3–5	0.648	0.553	——	——
Sept. 5–7	0.292	0.254	——	——
Sept. 7–9	0.175	0.153	——	——
Sept. 9–11	0.257	0.224	0.361	0.311
Sept. 11–13	0.273	0.227	0.406	0.312
Sept. 13–15	0.178	0.145	0.226	0.181
Sept. 15–17	0.178	0.154	0.229	0.176
Average	0.274	0.238	0.332	0.274
Percentage reduction in transpiration		13.1		17.5
Odds	2,499:1		2,499:1	

*The leaves were measured on Aug. 30, Sept. 3, 7, 11 and 15.

unit of surface area than do the healthy plants. These data are presented in Tables 5 and 6. In the first experiment the mosaic-infected plants in the crocks using the Livingston auto-irrigators transpired 12.1 per cent less per square centimeter of leaf surface than the healthy plants; while in the same experiment, but in the porous pot auto-irrigators, the mosaic-infected plants transpired 8.3 per cent less than the healthy plants (Table 5).

The results of the second and third experiments presented in Table 6 agree with the findings of the first experiment, namely, that mosaic-infected plants transpire less per unit of surface area than do healthy plants. In the porous pot auto-irrigators the mosaic-infected plants transpired 13.1 per cent less than the healthy plants and in the tared crocks weighed with balances the mosaic-infected plants transpired 17.5 per cent less than the healthy plants. The amount of water transpired per gram of dry matter also was calculated for the last reading from these two experiments. On this basis the mosaic-infected plants transpired 12.4 per cent less than did the healthy plants (Table 7).

TABLE 7.—CUBIC CENTIMETERS OF WATER TRANSPIRED PER GRAM OF DRY MATTER FROM MOSAIC-INFECTED AND HEALTHY PLANTS.

PLANT NO.	WATER TRANSPIRED PER GRAM OF DRY MATTER, CC	
	Healthy	Mosaic
	Experiment 2	
1	51.62	45.45
2	48.46	39.93
3	44.75	42.08
4	47.36	57.89
5	41.48	42.40
6	50.24	43.76
	Experiment 3	
1	56.30	38.53
2	53.45	45.34
3	64.30	45.21
4	63.48	50.18
5	61.64	59.91
Average	53.01	46.43
Percentage reduction in transpiration		12.4
Odds		66:1

The results obtained by the cobalt chloride paper clips on the rate of transpiration from mosaic-infected and healthy leaves confirm

those obtained with the two types of auto-irrigators and balances. Leaves with early symptoms, that is the first leaves to develop symptoms after inoculation, transpired 65.3 per cent more slowly than did the healthy leaves (Table 8), while leaves with typical symptoms transpired only 17.8 per cent more slowly than did the healthy leaves (Table 9). No significant difference was obtained in the rate of transpiration from leaves which had been fully formed before infection took place. Comparisons in the rate of transpiration between the light and dark areas of mosaic-infected leaves showed that the light areas transpired 18.6 per cent more slowly than did the dark areas (Table 10).

The fact that healthy bean leaves are injured more quickly than diseased leaves during periods of high transpiration further indicates that healthy plants transpire faster than do mosaic-infected plants.

Factors involved in variable transpiration.—The data presented in the preceding paragraphs show that mosaic increases the rate of transpiration from pods and decreases the rate from the leaves. This necessitated some explanation for the contradictory results. At first the fact that the pods were detached while the leaves were attached to the plant was considered as a possible explanation. This did not prove to be the case, however. In laboratory experiments, detached mosaic-infected leaves transpired more slowly than detached healthy

TABLE 8.—THE EFFECT OF MOSAIC ON TRANSPIRATION OF LEAVES WITH EARLY SYMPTOMS AS DETERMINED BY THE COBALT CHLORIDE PAPER METHOD.

LEAF NO.	TIME IN SECONDS TO CHANGE THE COBALT CHLORIDE PAPERS FROM BLUE TO BLUISH PINK	
	Healthy	Mosaic
1	73	256
2	51	81
3	50	152
4	45	144
5	75	107
6	60	415
7	72	168
8	65	95
9	64	280
10	80	132
Average	63.5	183.0
Percentage reduction in transpiration		65.3
Odds		416:1

TABLE 9.—THE EFFECT OF MOSAIC ON TRANSPIRATION OF LEAVES WITH TYPICAL SYMPTOMS AS DETERMINED BY THE COBALT CHLORIDE PAPER METHOD.

LEAF NO.	TIME IN SECONDS TO CHANGE THE COBALT CHLORIDE PAPERS FROM BLUE TO BLUISH PINK	
	Healthy	Mosaic
1	54	70
2	108	139
3	72	87
4	165	146
5	120	194
6	105	133
7	95	98
8	85	95
9	82	110
10	76	85
11	80	111
12	84	104
13	77	90
Average	92.5	112.5
Percentage reduction in transpiration		17.8
Odds		365:1

TABLE 10.—THE RATE OF TRANSPIRATION FROM DARK AND LIGHT AREAS OF MOSAIC-INFECTED LEAVES.

LEAF NO.	TIME IN SECONDS TO CHANGE THE COBALT CHLORIDE PAPERS FROM BLUE TO BLUISH PINK	
	Dark areas	Light areas
1	71	100
2	109	107
3	107	130
4	60	94
5	61	78
6	65	75
7	95	118
8	76	90
Average	80.5	99
Percentage reduction in transpiration		18.6
Odds		832:1

leaves. The assumption was drawn, therefore, that the same difference probably exists between attached mosaic-infected and healthy pods as exists between detached ones.

Various explanations summarized briefly by Graf-Marin (14) have been advanced to account for the effect of fungus diseases on the transpiration rate. In some cases the rate is increased, while in other cases the rate is decreased. In the case of leafroll of potatoes Krüger (21) states that the abnormal transpiration rate is associated with abnormal stomatal behavior. The stomates, therefore, were considered as a possible factor in the change in the transpiration rate of mosaic-infected plants. Other factors were also considered, such as anatomy, water content, and osmotic relationships.

In the study of the leaf stomates, the method employed by Loftfield (28) was used. This method, in brief, consists of stripping off the epidermis and placing it immediately in a preserving solution of absolute alcohol. Five sets of samples were obtained by stripping the lower epidermis from near the apex of terminal leaflets of compound leaves. Each set included samples from healthy and from mosaic-infected plants. Four of the samples from mosaic-infected plants were taken from leaves showing early symptoms and the fifth sample from leaves showing typical symptoms. The samples were collected at intervals during a period of about 29 hours between the afternoon of one day and the evening of the following day.

The number of stomates was counted in three microscopic fields from each sample in order to determine the number of stomates per unit of surface area. Each field, 0.413 square millimeters in area, was taken from a different strip of epidermis.

The results, presented in Table 11, show that mosaic does not affect the number of stomates per unit of surface area, but that it does affect the number of stomates which are open. A greater percentage of the stomates was closed on the mosaic-infected plants than on the healthy plants regardless of whether the samples were collected in the early morning or during the course of the day.

The degree of stomatal opening was also considered in these studies. Stomates for this purpose were picked at random by moving the mechanical stage and selecting the stomate nearest the center of the field. Thirty stomates, 10 on each of three strips of epidermis, were measured with a filar micrometer from each sample. No significant difference was obtained in the width of the stomatal pore on the mosaic-infected and healthy leaves (Table 11). Only the open or partly open stomates, however, were considered in these measurements.

The results of these studies on leaf stomates show that the only consistent difference between the stomates on mosaic-infected and

TABLE 11.—EFFECT OF MOSAIC ON THE NUMBER AND OPENING OF LEAF STOMATES.

SAMPLE NO.	DATE OF COLLECTION	HEALTHY			MOSAIC		
		Average number stomates per field*	Per cent of stomates closed	Average width of pore in μ†	Average number stomates per field*	Per cent of stomates closed	Average width of pore in μ†
1‡.....	Oct. 4, 2:30 p.m.	34.7	16.4	2.3	32.3	36.1	3.2
2.....	Oct. 5, 7:45 a.m.	35.0	14.3	2.6	33.7	33.7	2.6
3.....	Oct. 5, 1:30 p.m.	35.0	44.8	2.5	38.3	68.7	1.9
4.....	Oct. 5, 7:30 p.m.	28.0	78.6	1.9	37.0	91.0	1.0
5.....	Oct. 5, 1:45 p.m.	34.3	44.7	2.6	24.3	54.8	2.6
Average		33.4	39.7	2.4	33.1	56.9	2.3

*The microscopic field had an area of 0.413 sq. mm.
†Only the open stomates were considered in the measurements.
‡Samples 1, 2, 3, and 4 were taken from leaves with early symptoms.

healthy leaves was in the number that were open. From 10 to 24 per cent fewer of the stomates were open on the mosaic-infected leaves, than on the healthy leaves. This difference in the number of stomates open probably accounts for the decreased transpiration rate from mosaic-infected leaves.

Since bean mosaic caused an abnormal behavior of leaf stomates, these were considered in a possible explanation for the increased rate of transpiration from mosaic-infected pods. The strip method for studying stomates was found unsatisfactory for pods because it was difficult to strip off the epidermis. The method employed by Long and Clements (29) was then tried. Briefly it consists of making a print of the stomates by placing a drop of collodion on the pod. As soon as the collodion is dry the film is removed and examined under the microscope. The method was only partially successful because of the tomentose condition of the pods.

No consistent difference appeared to exist, however, in the number of stomates or in the percentage of stomates closed on the pods from healthy and mosaic-infected plants. A greater difference appeared to exist between pods of the same sample than between the healthy and mosaic-infected pods.

A study of the effect of bean mosaic on the anatomy of bean pods

was made from free-hand and microtome sections, but no significant difference was noted in the gross anatomy of the pods.

Hydrosis of the pods may frequently be a factor in increasing the rate of transpiration from mosaic-infected pods, but in cases in which the mosaic-infected pods show no symptoms of hydrosis, it obviously cannot be advanced as an explanation for the increased rate of transpiration. There is a possibility that the disease may affect the cells and protoplasts so that they are unable to retain the water, and consequently increase the transpiration rate.

The fact that plants with a high osmotic concentration are better able than plants with a low osmotic concentration to obtain and hold water under ordinary conditions may explain why mosaic-infected pods transpire faster than do healthy pods. In another part of this paper it is demonstrated that mosaic-infected plants have a lower percentage of dry matter than do healthy plants, therefore it is assumed that mosaic-infected plants also have a lower osmotic concentration than do healthy plants. If this assumption is correct, then the healthy pods probably are better able to obtain and hold water than are mosaic-infected pods.

REPRODUCTION

Some virus diseases induce complete sterility in plants, others induce a partial sterility, while still others have little or no effect on the reproductive process. Frequently the reproductive organs of virus-infected plants are markedly deformed or changed in color. Virus infection also may affect time of maturity.

The effect of bean mosaic on reproduction of the bean plant depends largely on the variety. Observations on this point have been made by Reddick and Stewart (41), Pierce and Hungerford (37), Fajardo (11), and Nelson (35). No carefully controlled experiments, however, have been conducted to determine the effect of bean mosaic on the reproduction of the plant.

Deformation of flowers.—Deformation of the floral organs in various plants infected with virus diseases have been reported in the literature, in fact so-called new varieties of tulips have been originated by infection with a virus disease (5).

Bean mosaic increases the number and percentage of misshapen blooms on the plant as shown in Table 12. Altho they may be deformed, all parts of the flower are usually present. In some cases only the calyx or corolla is deformed, while in other cases all parts of the flower are distorted. Some of the deformities of bean flowers are shown in Fig. 6.

FIG. 6.—DEFORMED BLOOMS PRODUCED ON MOSAIC-INFECTED PLANTS. The two blooms on the left are normal.

TABLE 12.—EFFECT OF MOSAIC ON THE DEFORMATION OF BLOOMS.

	HEALTHY*	MOSAIC†
Total number blooms	2,619	1,620
Number blooms per plant	124.7	85.3
Total deformed blooms	37	180
Deformed blooms per plant	1.8	9.5
Percentage deformed blooms per plant	1.4	11.1

*21 plants.
†19 plants.

Deformation of pods.—Little or no attention has been given to the effect of mosaic on pod shape. Since this is of importance to the quality of canned beans and to the mechanics of canning them, a study was made of the effect of mosaic on the curvature of the pod and on the production of what the trade calls "polliwogs", i.e., one-seeded pods.

Pods from mosaic-infected and healthy plants growing in the field or greenhouse were classified into four groups[6], namely, good, including those pods which were straight or very slightly curved; medium, including pods which were curved enough to interfere slightly with the mechanics of snipping the ends and cutting in the cannery; bad, including both polliwogs and pods which were curved so that they were difficult to handle; and a group consisting only of polliwogs.

In one experiment pods picked at random from mosaic-infected and healthy plants in a commercial field were graded from the first and

[6] This classification had the approval of several commercial canners.

second pickings. Some data were also obtained from greenhouse and field experiments in which the blooms were tagged as they appeared.

A summary of these studies, presented in Tables 13 and 14, clearly demonstrates that mosaic markedly increases the curvature of bean pods. Mosaic, however, does not appear to influence the production of "polliwogs" because this phenomenon seems to occur as frequently on healthy plants as on mosaic-infected plants, at least in the variety Stringless Green Pod Refugee. This is contrary to the observation made by Nelson (35) who states that "in many cases only one very large seed is matured in a pod."

Mosaic, however, is not the only cause of the curvature of bean pods. Any condition which interferes with the growth of the bean plant probably will cause increased curvature of the pods. Pods on plants affected with red spider or mildew or on plants growing in soil with low fertility or a deficiency in moisture are almost invariably more crooked than those produced on plants growing under favorable conditions. The fact that mosaic-infected plants are handicapped by the virus may partially explain why the pods on mosaic-infected plants are more curved than pods on healthy plants.

In cage 1 of the experiment of 1933, described on page 32, pods were picked as they reached marketable size. The percentage of medium and bad pods increased with the number of pickings on both the healthy and mosaic-infected plants (Table 14). This phenomenon is probably related in some way to the growth rate.

Hydrosis of pods.—A condition which has been termed hydrosis because of the water-soaked appearance of the pods, and which apparently is associated with bean mosaic, was noticed by Harrison, Horsfall, and Burkholder (15) in 1932. The affected pods are usually glassy in appearance and lack the pubescence of a healthy pod. Occasionally the pods exhibit an uneven and pimply condition which somewhat resembles edema. Sometimes whole sections of the pods display a dark green water-soaked appearance which has given rise to the name hydrosis. Closer observation reveals that most of the pods have small irregular water-soaked lesions forming an indefinite net-work of hydrotic tissue. The appearance of hydrotic pods is illustrated in Fig. 7. Hydrotic pods assume a much darker color in the can than do healthy pods, and consequently are a cause of some of the inferior quality found in freshly canned beans.

When hydrosis was first observed the cause of the malady was obscure. Several theories were advanced to account for the trouble. Some canners and farmers thought it was associated with seed source;

TABLE 13.—EFFECT OF MOSAIC ON PRODUCTION OF CURVED AND MISSHAPEN PODS.

	HEALTHY					MOSAIC				
	Total number of pods	Percentage of pods that were				Total number of pods	Percentage of pods that were			
		Good	Medium	Bad	Polliwogs		Good	Medium	Bad	Polliwogs
				Geneva Experiments						
Field tagging	736	45.5	34.0	20.5	11.0	723	7.7	67.4	24.9	9.8
Greenhouse tagging	908	62.0	23.1	14.9	7.7	608	26.3	31.7	41.9	25.4
				Commercial Field						
First picking	300	71.7	20.0	8.3	0.0	300	61.7	22.7	15.7	0.0
Second picking	300	85.7	12.3	2.0	0.0	300	57.7	27.3	15.0	3.7

TABLE 14.—EFFECT OF MOSAIC AND NUMBER OF PICKINGS ON THE PRODUCTION OF CURVED AND MISSHAPEN PODS, CAGE EXPERIMENT, 1933.

DATE OF PICKING	HEALTHY					MOSAIC				
	Average number of pods per plant	Percentage of pods that were				Average number of pods per plant	Percentage of pods that were			
		Good	Medium	Bad	Polliwogs		Good	Medium	Bad	Polliwogs
Aug. 19	6.1	93.4	3.3	3.3	0.0					
Aug. 25	6.8	80.9	11.8	7.4	5.1					
Aug. 28	10.5	77.0	12.4	10.5	7.7	0.5	62.5	37.5	0.0	0.0
Sept. 1	7.5	70.3	23.6	6.1	4.1	4.2	52.5	45.0	2.5	2.5
Sept. 6	9.9	54.5	40.4	5.1	3.0	10.4	37.9	55.6	6.6	1.0
Sept. 11	1.8	25.7	51.4	22.9	5.7	5.9	32.4	55.9	11.7	1.8
Sept. 18						6.4	42.5	35.8	21.7	6.7
Sept. 25						4.3	6.2	60.0	33.8	12.5

FIG. 7.—HYDROSIS OF BEAN PODS.

Left, normal pod; right, pod obtained from a plant infected during the blooming period.

others that it was due to disturbances in soil moisture, soil temperature, type of soil, or other environmental conditions. Since the first occurrence, hydrosis has been observed many times both in the field and in the greenhouse but always on mosaic-infected plants. The pods on mosaic-infected plants do not always exhibit symptoms of hydrosis and occasionally pods on healthy appearing plants will show the characteristic water-soaked condition, but such apparently "healthy" plants always have later on developed the typical symptoms of mosaic.

Observations were made during the summers of 1933 and 1934 on the severity of hydrosis in various seed lots planted at Geneva and in other parts of the State. Over 100 samples were examined from time to time, but at no time was it possible to distinguish any significant difference in the occurrence of hydrosis in the different seed lots.

The fact that environmental conditions in some way play a part in the appearance of hydrosis was observed in an experiment made to determine the effect of mosaic on the production of blooms and pods. Pods in the first two pickings from seed-infected plants showed no symptoms of hydrosis, while a large percentage of the pods in

the third picking had distinct symptoms of hydrosis. The percentage of pods affected and the severity decreased in the later pickings. In the sixth and last picking only a few affected pods were observed and these had developed only slight symptoms. Since a period of high humidity prevailed just prior to the third picking, experiments were conducted in an effort to produce hydrosis experimentally with a high humidity. Mosaic-infected plants in the greenhouse and in the field were subjected to varying soil moistures and air humidities, but hydrosis developed to about the same extent in all lots.

The stage of growth at which the plant is infected has a marked influence on the severity of hydrosis. This observation was made in an experiment in which plants grown from a pure line of seed infected with mosaic were examined at approximately weekly intervals for the development of mosaic. At each examination those plants which had developed symptoms since the previous examination were staked. A distinct difference in the severity of hydrosis on the different plants was observed in the first pickings. Consequently, at the fourth picking the plants were classified according to the severity of hydrosis on the pods. The classification was as follows: *None,* if no symptoms were detected on any of the pods; *slight,* if the symptoms on one or more pods were just in evidence; *medium,* if the symptoms on one or more pods were distinct but not disfiguring; and *severe,* if one or more pods were disfigured from hydrosis.

In order to reduce the readings to a single figure a weighted average was calculated as follows: The number of plants in each group with no symptoms was multiplied by 1, the number with slight symptoms by 2, the number with medium symptoms by 3, and the number with severe symptoms by 4. These products were summed and the total divided by the number of plants in each group, thus an index of severity was obtained. The results, presented in Table 15, indicate rather clearly that hydrosis is most severe on recently infected plants. A total of 335 plants was used in the investigation.

Effect of mosaic on flower production.—The effect of mosaic on the time of flowering was determined in experiments conducted in 1933 on the variety Stringless Green Pod Refugee. Seeds from a pure line infected with mosaic were planted on June 15 in two large cheesecloth cages. Each cage was 5 feet wide, 32 feet long, and 3.5 feet high. A good grade of cheesecloth was used to exclude insects. When the plants were large enough for mosaic to be detected they were thinned so that each cage had one row of healthy and one row of diseased plants, with 40 plants in each row.

TABLE 15.—EFFECT OF TIME OF INFECTION ON SEVERITY OF HYDROSIS ON PODS.

STAGE OF GROWTH WHEN SYMPTOMS FIRST DEVELOPED	NUMBER OF PLANTS EXAMINED	NUMBER OF PLANTS WITH HYDROTIC PODS				INDEX OF SEVERITY
		No hydrosis	Slight hydrosis	Medium hydrosis	Severe hydrosis	
		Experiment I				
Seedling	9	0	8	1	0	2.11
6th to 7th leaf	10	1	7	1	1	2.20
Blooming	30	1	18	10	1	2.37
Small pod	15	4	11	0	0	1.73
First picking	14	4	6	2	2	2.14
Third picking	19	0	0	4	15	3.79
Fourth picking	6	0	0	2	4	3.67
Healthy	7	7	0	0	0	1.00
		Experiment II				
Seedling	44	2	34	7	1	2.16
3rd to 4th leaf	46	1	31	14	0	2.28
6th to 7th leaf	43	2	29	11	1	2.25
Blooming	19	4	13	2	0	1.89
Small pod	15	6	7	1	1	1.80
First picking	6	0	1	0	5	3.67
Second picking	21	0	1	0	20	3.90
Third picking	18	0	6	7	5	2.94
Healthy	13	13	0	0	0	1.00

The blooms were removed and counted daily on one-half of the healthy and one-half of the diseased plants in Cage 1, while the blooms were counted but not removed on the remainder of the plants. In the latter case the pods were removed as soon as they reached marketable size. In Cage 2 the blooms were tagged daily.

The results from these cage experiments demonstrate that mosaic delayed the blooming period of the plants approximately two weeks (Fig. 8). The same phenomenon was observed in the field and in other experiments, but the delay in these cases was usually from 7 to 10 days. In general, these results are in agreement with the observations of Pierce and Hungerford (37) and Fajardo (11).

The data in Fig. 8 reveal another interesting point, *viz.*, that the Stringless Green Pod Refugee may be made to grow almost indefinitely by removing the blooms as they are produced. This is true for both mosaic-infected and healthy plants. At the conclusion of the experiment, 68 days after the first bloom was produced, the plants were still blooming profusely.

The blooming date of the bean plant is influenced also by the time of infection with the mosaic virus. The writer observed that the blooming date of plants infected in the seedling stages was about the

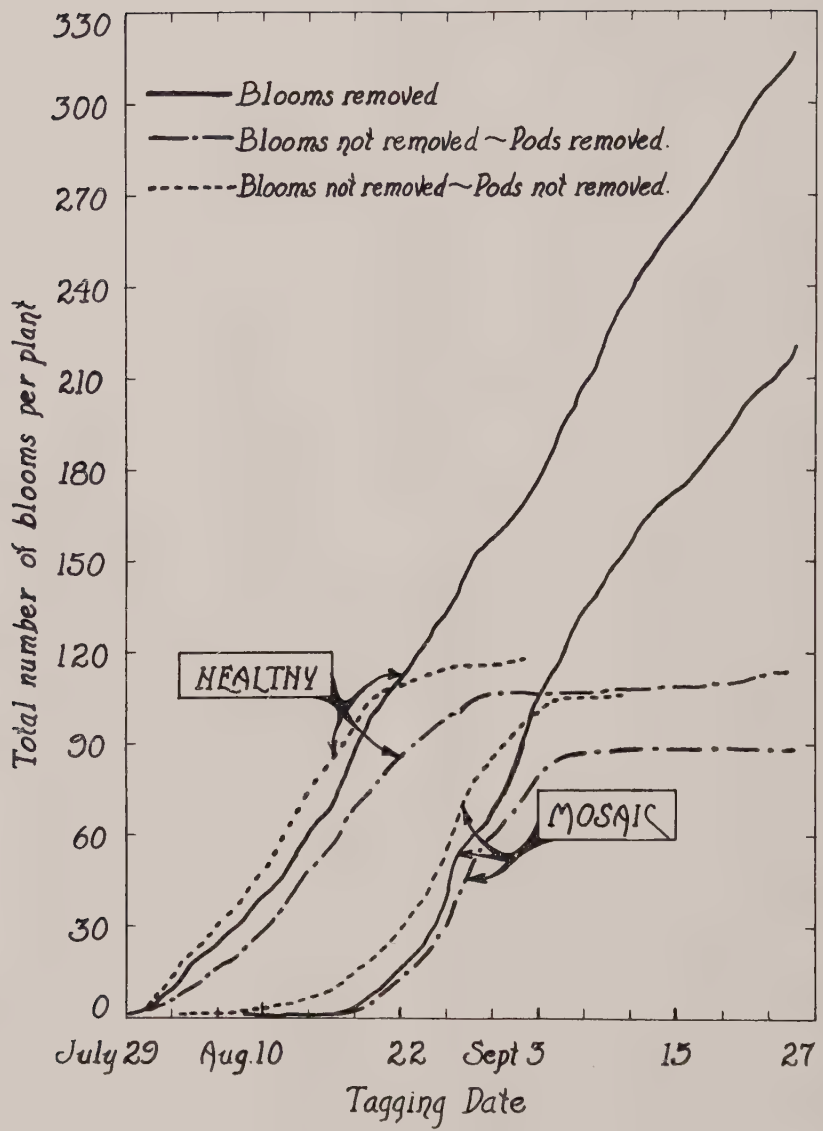

FIG. 8.—INCREMENT CURVES SHOWING THE EFFECT OF MOSAIC ON THE PRODUCTION OF BLOOMS.

Plants grown in large cheesecloth cages in the field in 1933.

same as that of plants from infected seeds. However, if infection did not occur until just prior to the blooming period, the delay in blooming was even more pronounced. If, on the other hand, infection took place during the blooming period, bloom production ceased for several days, this lapse being followed usually by another blooming period.

Bean mosaic may affect flower production in other ways than by delaying the blooming period, the nature of the effect depending to a large extent on the variety. Nelson (35) states that mosaic-infected plants usually fail to produce the normal number of blooms. Pierce and Hungerford (37), Fajardo (11), and Nelson (35) report that the blooms shed more freely from mosaic-infected plants than from healthy plants. No data are presented, however, by these investigators.

The effect of mosaic on the production and shedding of blooms was determined in field experiments just described on pages 32 and 33, and also in greenhouse experiments conducted during the summers of 1933 and 1934. For the greenhouse experiments a pure line of seed infected with mosaic was planted in electrically pasteurized soil. The young seedlings were thinned as soon as mosaic could be detected, so that half of the plants were healthy and half infected with the mosaic virus. In the 1933 experiments, the seed for the healthy and for the mosaic-infected plants was planted at the same time; whereas in 1934 the seed for the healthy plants was planted 7 days later than the seed for the mosaic-infected plants so that both bloomed over approximately the same period of time.

In the 1933 greenhouse experiment the blooms were tagged as they opened, while in the 1934 greenhouse experiment the blooms were counted daily. The results from these experiments showed that healthy plants produced more blooms per plant than did the mosaic-infected plants under both greenhouse and field conditions (Table 16 and Fig. 8). Mosaic, however, appears to have a marked influence on the setting of blooms under certain conditions. In the field, no significant difference was obtained in the percentage of blooms set on healthy and mosaic-infected plants, while in the greenhouse, in 1933, 41.1 per cent more of the blooms set on the healthy plants than on the mosaic-infected plants, and in 1934 the difference was 20.3 per cent, likewise in favor of the healthy plants.

The data presented in Table 16 also show that the removal of pods as soon as they reach marketable size increased the percentage of blooms set in like proportions on both mosaic-infected and healthy

TABLE 16.—EFFECT OF MOSAIC ON PRODUCTION AND SETTING OF BLOOMS.

	HEALTHY		MOSAIC		PER CENT DECREASE IN BLOOMS SET	ODDS
	Average number of blooms per plant	Percentage of blooms set	Average number of blooms per plant	Percentage of blooms set		
1933 Experiments						
Greenhouse, pods left on until mature	53.9	34.8	43.7	20.5	41.1	369:1
Field cage, pods left on until mature	115.8	16.3	107.4	17.3	—6.1	Not significant
Field cage, pods removed at marketable size	114.1	37.9	88.6	35.4	6.6	Not significant
Field cage, blooms removed daily	315.8		221.4			
1934 Experiments						
Greenhouse, pods left on until mature	38.4	41.9	35.6	33.2	20.3	499:1

plants. The two field experiments in 1933 were conducted under the same conditions, except that the pods were removed when they became of marketable size in one case and were left attached to the plant in the other case.

Another point of interest observed in these experiments was that, altho healthy plants produced more blooms per plant than did the mosaic-infected plants, this may have been due to the fact that the healthy plants were larger. As a matter of fact, the infected plants under greenhouse conditions produced 17.3 per cent more blooms per gram of dry matter than did the healthy plants (Table 17). No significant difference was obtained in the number of blooms produced per gram of dry matter on mosaic-infected and healthy plants in the field, however. The dry matter determinations were made on the plants from cage 1 in the 1933 field experiments and on the plants in the 1934 greenhouse experiment. For details see page 39.

The difference between the greenhouse-grown and the field-grown plants requires explanation. Ecological conditions in the greenhouse were made favorable for the setting of pods so that differences due to disease showed plainly. The healthy plants in the field experi-

TABLE 17.—THE NUMBER OF BLOOMS PRODUCED PER GRAM OF DRY MATTER ON MOSAIC-INFECTED AND HEALTHY PLANTS, GREENHOUSE EXPERIMENT 1934.

SECTION	AVERAGE NUMBER OF BLOOMS PER GRAM OF DRY MATTER	
	Healthy	Mosaic
1	1.93	2.45
3	2.04	2.46
4	1.87	2.37
5	1.78	2.36
6	2.07	2.40
7	1.88	1.84
8	2.14	2.23
Average	1.96	2.30
Per cent increase		17.3
Odds		250:1

ments commenced to bloom several days ahead of the mosaic-infected plants, as would be expected from the delaying effect of the disease. Hot, dry weather that prevailed during this period was not conducive to pod setting, however. The drought was broken shortly after the mosaic-infected plants started to bloom, so that they set the majority of their pods under much more favorable circumstances than did the healthy plants.

Effect of mosaic on pod production.—The effect of mosaic on the setting of pods has been discussed in the previous section. The aspect of podding that has the most practical interest is the yield. Yields, therefore, were obtained for seed-infected plants and for healthy plants both in the greenhouse and in the field. In the greenhouse the plants were grown in electrically pasteurized soil and shaded by cheesecloth from the hot rays of the sun, while the plants in the field were grown in cheesecloth cages of various sizes. A summary of these results, which are presented in Table 18, gives some indication of the reduction in total yield due to mosaic.

Additional data on yield reduction by mosaic were obtained in a 5-acre commercial field at Cato, New York, in 1934. Three acres of this field were planted with mosaic-free seed and 2 acres with commercial seed. At the time of harvest the incidence of mosaic ranged from 3 to 7 per cent in the former portion of the field and from 15 to 30 per cent in the latter portion. The yield records obtained thru the courtesy of the Red Creek Canning Company showed that the portion of the field planted with commercial seed yielded 3.48 tons per acre

TABLE 18.—EFFECT OF MOSAIC ON YIELD FROM PLANTS GROWN IN THE FIELD AND IN THE GREENHOUSE.

EXPERIMENT	HEALTHY		MOSAIC		PERCENTAGE DECREASE	REMARKS
	Number of plants	Average number pods per plant	Number of plants	Average number pods per plant		
Field, 1933..	34	7.3	34	3.1	57.5	Cages small; plants crowded; pods left on until mature
Field, 1933..	20	43.2	19	31.4	27.3	Cages large; plants not crowded; pods removed at marketable size
Field. 1933..	39	18.9	39	18.5	2.1	Cages large; plants not crowded; pods left on until mature
Greenhouse, 1933	51	17.7	71	9.0	49.2	Plants not crowded; pods left on until mature
Greenhouse, 1934.....	27	16.2	45	11.9	26.5	Plants not crowded; pods left on until mature

as compared to 4.15 tons per acre for the portion planted with mosaic-free seed, or a decrease in yield of 16.1 per cent. Most of this difference in yield was due to mosaic, altho part of it may have been due to soil conditions.

Another point of interest is that the portion of the field planted with mosaic-free seed was picked only four times as compared with five times for the portion planted with commercial seed. The extra picking was due primarily to the fact that mosaic delays the blooming and consequently the pod-setting period.

These findings agree with those of Fajardo (11) and others to the effect that mosaic reduces the total yield per plant for the variety Stringless Green Pod Refugee.

Plant size was not considered in the discussion in the preceding paragraphs on the effect of mosaic on the yield per plant. Since plant size is an important factor in governing the yield per plant, and since mosaic-infected plants are smaller than healthy plants, the yield would naturally be less on the mosaic-infected plants than on the healthy plants. Therefore, to determine accurately the effect of mosaic on yield, the pod production from mosaic-infected and healthy

plants of the same size should be compared. This was done in two experiments, one conducted in the field in 1933 and another conducted in the greenhouse in 1934. The dry weight of the plant without roots was used as a measure of the plant size, and the number of pods was taken as a measure of the yield. The plants were dried in individual paper sacks in a large steam-heated drying room.

In the 1933 field experiment, which is described on page 32, the pods were not included in the dry weight of the plant since they were picked at frequent intervals. The dry weights of the plants were obtained after the last picking of the pods. In the 1934 greenhouse experiment, which is described on page 35, the dry weights of the plants were obtained about 2 weeks after the plants ceased blooming so that the pods were included in the dry weight.

The results of these two experiments are presented in the form of graphs in Figs. 9 and 10. The number of pods each plant produced is plotted against the dry weight of the plant. From these graphs it is evident that mosaic-infected and healthy plants of the same size produce approximately the same number of pods. In other words, a straight line relationship apparently exists between plant size and the number of pods produced. Dwarfing of the plant, therefore, is the chief reason for the decreased yield on mosaic-infected plants.

Effect of mosaic on pod growth.—In order to determine whether mosaic influences the growth rate of pods, pods of known age were

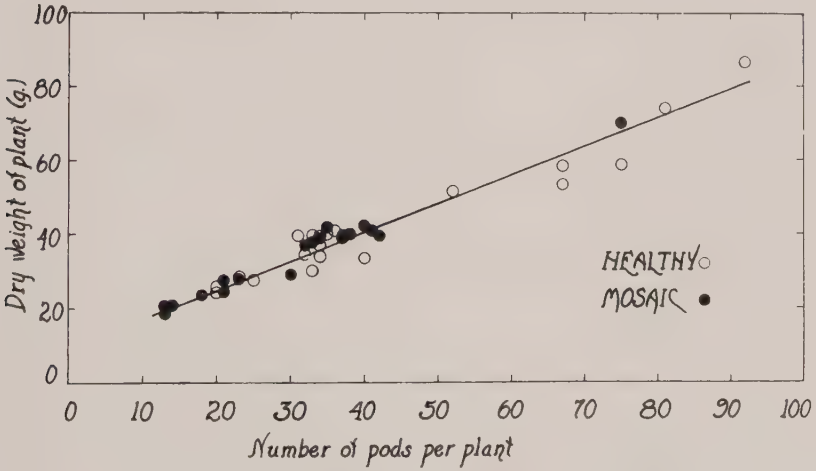

Fig. 9.—Relation of Pod Production to Dry Weight from Mosaic-infected and Healthy Plants.

Plants grown in a large cheesecloth cage in the field in 1933.

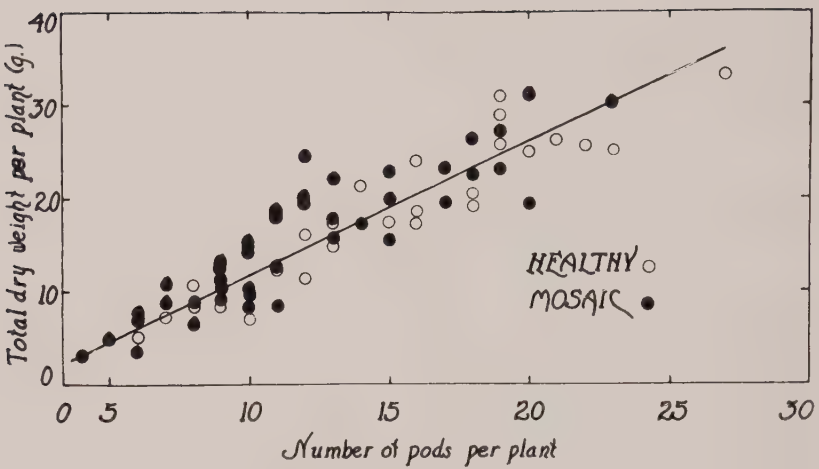

FIG. 10.—RELATION OF POD PRODUCTION TO DRY WEIGHT FROM MOSAIC-INFECTED AND HEALTHY PLANTS.

Plants grown in electrically pasteurized soil in the greenhouse in 1934.

examined. No significant difference was noticed in the rate of growth of mosaic-infected and healthy pods, since sometimes the infected pods were larger than healthy pods of the same age and sometimes the reverse was true. The position of the pod on the plant seemed to be of more importance in determining its growth rate than was the presence or absence of the virus in the plant.

Effect of mosaic on maturity.—The results of the experiments on the effect of mosaic on reproduction clearly show that the delayed maturity of the mosaic-infected plants is due primarily to a delay in the blooming period and not to a shedding of the early formed flowers or to a more rapid growth rate of the pods on healthy plants. An interesting point in connection with delayed maturation of diseased plants is that removal of green plants in the fall becomes a simple method of eliminating diseased seed from a seed lot. This is only possible, however, in fields where some of the plants escape infection before blooming.

EFFECT OF MOSAIC ON QUALITY

Bean mosaic is a direct cause of much of the poor quality of pods from the variety Stringless Green Pod Refugee. It causes crooked and curved pods, rough and pimply pods, and hydrotic pods, as has been shown in the preceding sections. Furthermore, when pods from mosaic-infected plants are compared with pods from healthy plants,

they are much darker in color. This condition is more noticeable in mass than when individual pods are compared. The darkened condition of the pods remains after going thru the commercial canning process. Thus, when mosaic-infected and healthy pods are canned together in the same can some of the pods will frequently be darker than others. No distinct differences, however, have been noticed in the flavor or texture of mosaic-infected and healthy pods after they have been canned.

EFFECT OF ENVIRONMENT ON EXPRESSION OF MOSAIC SYMPTOMS

Knowledge of factors governing the expression and masking of virus disease symptoms is of extreme importance to the research worker. In the case of bean mosaic, various workers (*7*, 10, 20, 40) have observed that high temperatures and long days favor the expression of symptoms, while low temperatures and short days favor the masking of symptoms. The experimental results of Pierce and Hungerford (*37*) and of Fajardo (11) in the main agree with the observations of other workers. Fajardo, however, states further that at temperatures from 28° to 32°C a partial masking of symptoms results.

The work of Pierce and Hungerford indicates that exposure to long days favors the expression of symptoms. Their experiments, however, were not very extensive. Furthermore, Pierce and Hungerford and Fajardo did not have accurate temperature control in their experiments. Experiments were conducted by the writer, therefore, to study the effect of light and temperature on symptom expression of bean mosaic on the variety Stringless Green Pod Refugee.

Experiments.—Five separate experiments were conducted during the summers of 1933, 1934, and 1935 in a climate control laboratory designed by Dr. James G. Horsfall, in which both temperature and light were accurately controlled.

The laboratory consisted of a series of cubical chambers, each 4 x 4 x 4 feet within a refrigerated room in which the temperature was maintained uniformly, usually at 10°C. Each chamber was supplied separately with heat, thermostatically controlled. The temperature of the chambers varied little from the desired temperatures because of the constant room temperature. Sometimes the temperature rose as much as 2°C when the lamps were on and then fell back to the original temperature when the lamps were turned off. When the lamps were off the temperature variation was usually less than 0.5°C.

The light for each chamber was supplied by four 500-watt incandescant mazda lamps screened thru 1-inch of flowing water and controlled by an automatic time switch.

In the first experiment mosaic-infected seeds were planted in 6-inch pots in the greenhouse. The healthy plants were removed as soon as mosaic could be detected on the simple leaves of the infected plants. The mosaic-infected seedlings were then distributed in chambers maintained at 15°, 20°, and 25°C. Eight pots were placed in each chamber on a shelf which was 4 feet below two of the 500-watt lamps and given an 8-hour day.

The next three experiments varied from the first in only a few details. The young seedlings in the second experiment were given a 12-hour day, and the shelves were 2 feet below the lamps. For the third experiment 10 mosaic-infected seeds were planted in 1-gallon crocks and germinated at 25° C. After germination the crocks were distributed in 15°, 20°, 25°, and 30° C chambers and given a 10-hour day, the shelves in this case being 2.5 feet from the lamps. Eight crocks were placed in each chamber. The fourth experiment was a duplicate of the third except that the crocks were removed to the greenhouse after the germination of the seeds. Five days in the greenhouse were sufficient to overcome the extremely spindling growth which resulted in the third test.

In the fifth and final experiment the pots containing the mosaic-infected seedlings were subjected to different light intensities at 20°C by placing part of the plants on shelves 2.5 feet from the lamps and the remainder on the floor of the chamber 4 feet from the lamps and giving them a 12-hour day. The light intensity for the plants on the shelf varied from 800 to 1,200 foot-candles, while the light intensity for the plants on the floor was from 150 to 300 foot-candles.

The length of the treatment depended on the temperature of the chamber. In the 20° and 25°C chambers the exposures ranged from 14 to 18 days, while in the 15° and 30°C chambers the exposures ranged from 18 to 22 days. The plants were removed to the greenhouse for observation as soon as the new growth appeared.

Discussion of results.—The results of these experiments were in accord with the findings of Fajardo (11), namely, that high and low temperatures favor the masking of symptoms. The characteristic mosaic mottling was almost completely masked at 15° and at 30°C. In the 20° and 25°C chambers the mosaic mottling was distinct. The mosaic-infected plants under greenhouse conditions, however, produced a more distinct type of mottling.

Temperature also had a pronounced effect on the degree of rolling of both healthy and mosaic-infected leaves. No rolling was evident on any of the trifoliate leaves at 15°C. There was slight rolling of the mosaic-infected leaves at 20°, distinct rolling of the mosaic-infected leaves at 25°, and severe rolling of both healthy and mosaic-infected leaves at 30°C. It is interesting that the leaves of healthy plants were rolled in the 30° chamber. The rolling at 30° was so pronounced that mosaic-infected and healthy plants could not be distinguished except in a few cases. The low temperature of 15°C did not inhibit entirely the slight degree of downward rolling which frequently is observed on the simple leaves of seed-infected plants.

Symptom expression was not materially affected on leaves formed before placement in the chambers. The expression of symptoms was changed or inhibited only on leaves formed while in the temperature chambers.

Leaves in which the symptoms were masked did not develop symptoms when placed under favorable conditions in the greenhouse. However, if only a partial masking had resulted from the treatment, the symptoms were accentuated by exposure to favorable conditions.

Intensity of light apparently is an important factor in the development of bean mosaic symptoms, since a difference in the severity of the symptoms was noted. In the fifth experiment where a direct comparison was made between weak light and strong light, the mosaic mottling was almost completely masked in the weak light while it was fairly distinct in the strong light. Little or no rolling of the leaf margins occurred at the different light intensities, probably due to the fact that the temperature, 20°C, was not high enough for good leaf-rolling symptoms.

Length of day apparently is not as important a factor as temperature or intensity of light in the symptom expression of bean mosaic, since distinct symptoms developed in the 8-, 10-, and 12-hour days. They were slightly more pronounced, however, under the 12-hour day than under the 8-hour day. The lessened severity of the symptoms under the 8-hour day probably was due to the decreased intensity of the light, since the plants in that experiment were 4 feet from the source of light, whereas the plants in the other experiments were 2 or 2.5 feet from the light source.

The symptoms in no case were as pronounced as those that developed on the check plants in the greenhouse.

SUMMARY

This paper reports the results of investigations dealing with the physiological activities of healthy and mosaic-infected bean plants and certain properties of the bean mosaic virus. The studies were confined to the variety Stringless Green Pod Refugee.

The subjection of mosaic-infected seed to high temperatures (100°C for several hours), to formaldehyde fumes, and to X-rays failed to inactivate the virus or to eliminate the infected seeds. Flotation methods failed to show any significant difference between the specific gravity of healthy and mosaic-infected bean seed.

The percentage of dry matter in the tops of mosaic-infected plants from seedling to blooming stages is significantly less than in healthy plants.

Mosaic-infected bean leaves transpire significantly less per unit of surface area and per gram of dry weight than do healthy leaves. The same relationship holds true for detached leaves. However, pods from mosaic-infected plants transpire more rapidly per unit of surface area and per gram of fresh weight than healthy pods of the same size.

The reduced number of open stomates on the mosaic-infected leaves probably accounts for the decreased transpiration rate from mosaic-infected plants. No definite factor was found to account for the increased transpiration rate from mosaic-infected pods, but it probably is associated with the osmotic concentrations and the ability of the cells to hold water.

In experiments using cobalt chloride papers, the light green areas of mosaic-infected leaves were found to transpire significantly less than the dark green areas.

Bean mosaic causes an increase in aberrations of the reproductive organs. More of the blooms on infected plants are deformed, and infected pods have a distinct tendency to be more curved and misshapen than those on healthy plants. Furthermore, the pods on mosaic-infected plants frequently are rough and edematous, especially if the plants are infected in the blooming stage.

A condition which has been termed hydrosis because of the water-soaked appearance of the pods has been found to be constantly associated with bean mosaic under certain conditions. It has been definitely established that hydrosis is more severe on pods or plants infected in the blooming stage or just prior to the blooming stage than on plants infected in the seed or seedling stages. The rôle which ecological factors play in its development has not been determined.

Bean mosaic, on the average, causes a delay of several days in the blooming period of plants infected in the seed or seedling stages. However, if infection takes place during the blooming period, the plants cease blooming for a period of several days, this period usually being followed by another blooming period.

Mosaic-infected plants frequently produce more blooms per plant and per gram of dry matter than healthy plants; nevertheless, the yield in pods per gram of dry weight is approximately the same. The yield per plant on mosaic-infected plants, however, is materially reduced because of the stunting effect of mosaic.

The delayed maturity of the mosaic-infected plants is due to a delay in the blooming period and not to a shedding of the early formed blooms or to a differential growth rate of mosaic-infected and healthy pods.

Temperature is an important factor in the development of bean mosaic symptoms. The mosaic mottling was almost completely masked at both 15° and 30°C, while it was very distinct at both 20° and 25°C. At 30°C the leaves on both the healthy and mosaic-infected plants were so badly rolled that mosaic could not be detected. No rolling of the healthy leaves occurred at any of the other temperatures. The degree of rolling on the mosaic-infected leaves increased with a rise in temperature from no rolling on the compound leaves in the 15°C chamber to severe rolling in the 30°C chamber.

Intensity of light is an important factor in the development of mosaic symptoms, since they were more pronounced when grown at 2 feet from the source of light than when grown 4 feet from the source of light. Length of day, on the other hand, apparently is not an important factor in the development of bean mosaic symptoms since they developed approximately the same in an 8-hour day as in a 12-hour day.

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